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Comparison of Low and High Doses of Pentobarbital or Thiopental for Euthanasia of Isoflurane-anesthetized Pigs

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Barbiturate overdose is a common method for euthanizing pigs. However, barbiturates can cause tissue damage and may affect experimental results, so the minimal dose should be used. The minimal dose of barbiturate for euthanasia in pigs under isoflurane anesthesia has not yet been determined. In this study, we compared the effect of low and high doses of 2 barbiturates (pentobarbital, 30 or 60 mg/kg; thiopental, 20 and 40 mg/kg) on hemodynamic parameters and time to cardiac arrest in female pigs maintained on isoflurane. Acute decreases in blood pressure and end-tidal CO_2 occurred in all pigs shortly after administration of the barbiturate. However, these changes were not different between either of the high- and low dose groups. Cardiac arrest occurred significantly faster for high dose as compared with low dose thiopental groups, but this parameter was different between the 2 pentobarbital groups. The bispectral index fell immediately after dosing, in all pigs, but no significant differences were observed in the time needed to achieve 0 for the high or low-doses of either drug. In pigs maintained on isoflurane, a low dose of barbiturates is adequate for euthanasia and may result in less tissue damage.

Abbreviations and Acronyms: BIS, bispectral index; $EtCO_2$, end-tidal CO_2; HR, heart rate; MAP, mean arterial pressure

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Introduction

Euthanasia is an essential procedure in animal research. The method of euthanasia used must: (1) rapidly induce loss of consciousness and death, (2) be reliable, (3) minimize pain, fear and distress experienced by the animal, and (4) not interfere with study outcomes.[11] Moreover, the method of euthanasia should be simple enough to be performed effectively by any trained individual. According to *AVMA Guidelines for the Euthanasia of Animals*, the use of intravenous barbiturates is recommended as an acceptable method for euthanasia in pigs.[11] The most commonly used barbiturate is pentobarbital.[13] Thiopental, a short-acting barbiturate, is used occasionally.[1] Barbiturates are frequently used in anesthesia for their sedative-hypnotic properties;[17] intravenous doses of pentobarbital (20 to 40 mg/kg) or thiopental (6.6 to 25 mg/kg) can be used for this purpose, but higher doses may cause death due to cardiopulmonary depression and respiratory arrest.[11,17] Large doses of barbiturates may also cause tissue damage and artifacts that affect data interpretation.[7,16] Complications including hemorrhage, hemolysis, edema, and necrosis also occur frequently, especially in tissues close to the injection site, and these changes are time- and dose-dependent.[7] Euthanasia must reliably ensure humane death of the animal, but this goal must be accomplished using the minimal effective dose in order to limit interference with experimental results.

Pentobarbital has been used at a dose of 40 mg/kg or 80 mg/kg for euthanasia in pigs.[13] To our knowledge, no studies have investigated the dose of pentobarbital or thiopental needed for euthanasia in pigs that are already anesthetized with isoflurane. Isoflurane is a widely used inhalational anesthetic in veterinary medicine,[12] but it has cardiopulmonary depressant effects such as hypotension due to myocardial depression and decreased peripheral vascular resistance.[5] When used together, lower individual doses of isoflurane and barbiturates should be able to cause severe cardiopulmonary depression and death, and the use of lower doses could result in less tissue damage and reduce costs. We hypothesized that isoflurane-anesthetized pigs could be euthanized with doses of barbiturates that have similar efficacy as sedative-hypnotics. We tested this idea by evaluating the time to death course for pentobarbital and thiopental administered at low or high doses to pigs being maintained under general anesthesia with isoflurane.

Materials and Methods

Forty female pigs (12 Large White and 28 Landrace; 14.0 ± 2.5 wk old, 47.1 ± 6.9 kg that had been used to train doctors on medical procedures devices (coronary stenting, rotational atherectomy, 3D mapping of the heart, peripheral stenting, and coil insertions) were used for this study. This study was approved by the IACUC at the Institute for Advancing Science Miyazaki, Boston Scientific in Japan and was conducted in accordance with the guidelines of the Japanese Academic Council. All pigs were obtained from the same breeder (Farmtech, Miyazaki, Japan), group-housed under conventional conditions, fed commercial diets (MN Nikubuta Crumble, Marubeni Nisshin Feed, Tokyo, Japan) once daily, and provided water ad libitum. The animal room was set at a 14:10-h light:dark cycle (lights on 0700; lights off 2100). Temperature and humidity were adjusted to 20 to 26 °C and 30% to 80%, respectively. Toys such as balls and chains were provided for enrichment. After an acclimatization period of at least 7 d, pigs were used for training.

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All pigs were sedated with intramuscular administration of ketamine (5 mg/kg; Ketalar, Daiichi-Sankyo, Tokyo, Japan), medetomidine (0.06 mg/kg; Dorbene, Kyoritsu-Seiyaku, Tokyo, Japan), and butorphanol (0.2 mg/kg; Vetorphale, Meiji-Seika-Pharma, Tokyo, Japan), tracheally intubated, and placed on a ventilator (Fabius Tiro, Dräger, Lübeck, Germany). Positive-pressure ventilation was initiated, and anesthesia was maintained at 2% isoflurane (Isoflurane for animals, MSD Animal Health, Tokyo, Japan). A sheath introducer (Super Sheath, Medikit, Tokyo, Japan) was inserted into the left femoral artery and connected to a pressure transducer that was calibrated to zero at atmospheric pressure and connected to a medical monitor (CARESCAPE Monitor B650, GE Healthcare, Little Chalfont, United Kingdom). The mean arterial pressure (MAP), ECG, heart rate (HR), end-tidal CO_2 ($EtCO_2$), arterial oxygen saturation, and rectal temperature were all monitored. An arterial oxygen saturation probe was attached to the nasal mucosa. The ECG was obtained from the bipolar limb leads and V2 leads, and automatically calculated HR. Bispectral index (BIS) values were recorded using a BIS complete monitoring system (version 4.0, Medtronic, Dublin, Ireland). A BIS Quatro sensor was mounted on the left side of the pig's head, as previously described.[10] A sheath introducer (Super Sheath, Medikit) were inserted into the left femoral vein for medication and fluid administration during the training and were left in place for barbiturate administration during the study. Sterile saline (Karmipack Isotonic Sodium Chloride Solution, SD-Kawasumi Laboratories, Kanagawa, Japan) was infused at 5 mL/kg/h during the training.

After the training exercise was completed, the pigs were randomly assigned to receive an intravenous injection of 1 of the following (n = 10 per group): low-dose (30 mg/kg) or high-dose (60 mg/kg) pentobarbital (Somnopentyl, Kyoritsu-Seiyaku), or low dose (20 mg/kg) or high dose (40 mg/kg) thiopental (Ravonal, Nipro ES Pharma, Osaka, Japan). (Table 1).

Immediately after barbiturate injection the canula was flushed with saline flush and measurements were initiated. Each parameter was recorded every 5 s. In addition, the time of cardiac arrest and the time at which the BIS was 0 were recorded. Once cardiac arrest was confirmed, isoflurane inhalation and positive-pressure ventilation were discontinued. If cardiac arrest did not occur within 15 min of drug administration, additional doses of medication (30 mg/kg pentobarbital or 20 mg/kg thiopental) were planned.

A Welch t test was used to compare the low and high doses of each drug for each parameter. The Fisher exact test was used to compare the breeds and type of training. Statistical significance was set at $P < 0.05$.

Results

All pigs (n = 40) experienced cardiac arrest without the need for additional dosing. Figure 1 shows changes in biologic variables within 90 s after administration. Sharp decreases in MAP and $EtCO_2$ occurred immediately after barbiturate administration. Reductions in HR took relatively longer to occur. The time delays until cardiac arrest occurred are shown in Table 2. No significant differences were observed between the high- and low-dose pentobarbital groups, whereas cardiac arrest occurred significantly earlier in the high-dose thiopental group as compared with the low-dose group. In all cases, arterial oxygen saturation became difficult to measure during in the first minute after injection, and a value could not be displayed. The BIS value gradually decreased after barbiturate treatment and became 0 in all cases. The time taken to achieve a BIS of 0 is shown in Table 2, but no differences were observed between the high- and low-dose groups for either drug.

Discussion

Barbiturates are $GABA_A$ receptor agonists.[6] They appear to work by binding to $GABA_A$ receptors and enhancing the pharmacological effect of GABA by prolonging opening of transmembrane chloride ion channels, leading to hyperpolarization and central nervous system inhibition. Neurons of the cerebral cortex are more susceptible to these inhibitory effects than are the medullary systems concerned with circulation and respiration, but as the dose increases, these mechanisms lead to cardiopulmonary depression and death.[4]

BIS quantifies the level of consciousness from the EEG during the preceding 61.5 s.[8] In this study, the BIS value began to fall within 30 s after administration of either drug or dose, ending in a flat EEG; these changes were not different between the

Table 1. Main characteristics of 40 pigs. Values are the mean ± SD or number (n)

	Pentobarbital			Thiopental		
	High	Low	P[a]	High	Low	P[a]
n	10	10		10	10	
Age (weeks)	13.3 ± 3.7	13.2 ± 2.3	0.94	14.5 ± 2.1	14.8 ± 1.5	0.71
Body Weight (kg)	45.0 ± 8.2	44.6 ± 4.5	0.89	51.4 ± 8.7	47.5 ± 3.0	0.21
Breed (n)						
Large White	2	3	1.00	4	3	1.00
Landrace	8	7		6	7	
Training procedures performed (n)						
Coronary stent	1	1	0.84	2	1	1.00
Rotational atherectomy	4	3		4	4	
3D mapping of heart	3	5		3	3	
Peripheral stent and coil	2	1		1	2	
Total anesthetic time (min)	263 ± 55	274 ± 66	0.69	286 ± 53	307 ± 49	0.96
Rectal temperature at euthanasia (°C)	38.6 ± 0.5	38.4 ± 0.5	0.22	38.3 ± 0.4	38.3 ± 0.6	0.96

[a]The Welch t test was used to compare differences in age, body weight, total anesthetic time, and rectal temperature. A Fisher exact test was used to compare the differences between breeds and type of training.

2 doses used for each drug. In the present study, BIS values fell to 0 after administration of either drug, regardless of dose. and remained at 0. Thus, the EEG continued to flatten from 61.5 s before the BIS value becomes 0. Moreover, sharp decreases in $EtCO_2$ and MAP occurred in all groups immediately after barbiturate administration. These changes may relate to sudden drop in cardiac output, which has previously been shown to correlate with cardiac output.[15]

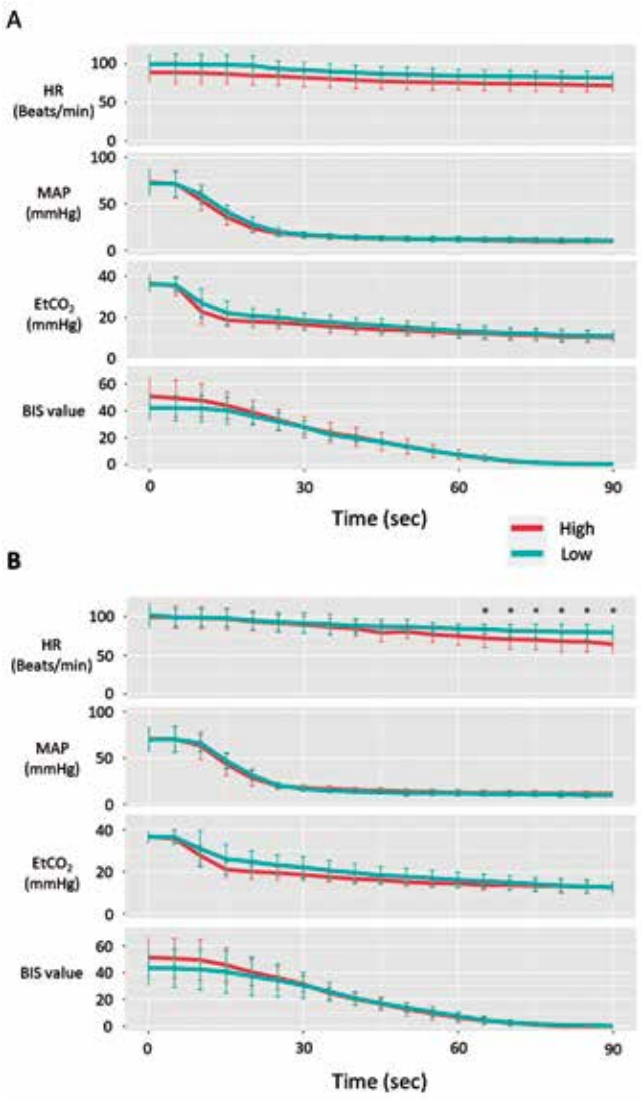

Figure 1. Changes in hemodynamic parameters, ventilation, and depth of anesthesia, during the 90 s after administration of pentobarbital (A) or thiopental (B). A Welch *t* test was used to compare high- and low-dose groups. *, $P \leq 0.05$. HR, heart rate; MAP, mean arterial pressure; $EtCO_2$, end-tidal CO_2; BIS, bispectral index

The decreases in MAP and $EtCO_2$ were the earliest changes in the parameters monitored, suggesting that barbiturates have a direct effect on the circulatory system and not just on the brain. The direct cardiac effects of barbiturates have been suggested based on pharmacological studies using blood-perfused, isolated canine hearts.[2,9,14] Studies of the cardiac effects of pentobarbital and thiopental have shown that these drugs have negative inotropic effects.[2,14] In the present study, the anesthetized pigs might also have experienced a strong negative inotropic effect at the barbiturate doses used. However, the decrease in HR and cardiac arrest took longer to occur than did the change in MAP. No differences in HR were detected between the high- and low-dose pentobarbital groups. A study using dogs showed that sinus rhythm disappeared when pentobarbital was injected directly into the sinus node artery.[9] However, when administered via the femoral vein, sinus rhythm was not affected, perhaps because of drug dilution in the circulation.[9]

For thiopental, the fall in HR was significantly faster for the high dose as compared with the low dose. A study using blood-perfused, isolated canine atria showed a negative chronotropic effect of thiopental when administered via the sinus node arteries.[14] Thiopental purportedly confers dose-dependent negative chronotropic effects even in the dosage ranges used in the current study.[14] Moreover, in our study, the time to cardiac arrest was significantly shorter in the high-dose thiopental group compared to the low dose group.

In all groups, intravenous administration of barbiturate resulted in a sudden drop in MAP and $EtCO_2$, followed by cardiac arrest several minutes later. The central and cardiovascular depressive effects of isoflurane were accentuated by concurrent use of barbiturates, which also have central and cardiovascular depressive effects.

This study had several limitations. First, male pigs were not evaluated. Sex differences have not been reported in studies of barbiturate use for euthanasia in monkeys.[7] However, to our knowledge, no studies have investigated such sex differences in pigs. Second, we did not perform necropsy. Whether artifact formation differs between the high- and low-dose groups warrants further investigation.

Our results indicate that low doses of the barbiturates pentobarbital and thiopental can be used for euthanasia in pigs maintained under general anesthesia. Because barbiturates cause dose dependent tissue toxicity,[7] they should be used for euthanasia at the lowest effective dose to avoid artifacts.[7] A previous recommendation was to perform euthanasia by using an overdose of barbiturate administered intravenously.[11] However, a relatively dose is sufficient to cause death in isoflurane-anesthetized pigs.[13] Using lower doses of barbiturates could contribute to reducing tissue damage, minimizing artifacts, reducing cost, and minimizing the impact of supply shortages, as happened in 2021 with pentobarbital.[3] Therefore,

Table 2. Time from drug administration to cardiac arrest (s) and time to BIS of 0 (s)

	Pentobarbital			Thiopental		
	High	Low	P^a	High	Low	P^a
Cardiac arrest (s)	337 ± 79	416 ± 86	0.051	208 ± 69	325 ± 77	0.002
BIS = 0 (s)	90 ± 34	83 ± 19	0.545	74 ± 6	85 ± 16	0.074

Values are presented as means ± SDs.
[a]The Welch *t* test was used to compare the high- and low-dose groups.
BIS, bispectral index

using a lower barbiturate dose for euthanasia of anesthetized pigs may have many advantages.

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